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First records of the seahorse *Hippocampus hippocampus* in Galician waters (NW Spain)

by

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Résumé. – Premier signalement de l'hippocampe *Hippocampus hippocampus* dans les eaux galiciennes (NO Espagne).

Le premier signalement de l'hippocampe à museau court *Hippocampus hippocampus* dans les eaux galiciennes (NO Espagne) est décrit sur la base de neuf spécimens adultes (118-183 mm LS) capturés lors d'inspections en plongée sous-marine entre 2010 et 2012. Les individus ont été identifiés selon les approches morphologiques et moléculaires. Ces données constituent d'importantes informations sur l'espèce, qui pourront être prises en compte lors de l'évaluation des populations et notamment à but de conservation.

Key words. – Syngnathidae - *Hippocampus hippocampus* - Seahorse - Galicia - Genetic identification - Conservation - New record.

All seahorse species (*Hippocampus* sp.) are included in the IUCN Red List (<http://www.iucnredlist.org>). The nomenclature and taxonomy of European seahorse taxa seem to be problematic, including two-three nominal names (Vasil'eva, 2007; Kuiter, 2009). In Kuiter (2009) alternative system, *Hippocampus guttulatus* would be classified as *Hippocampus hippocampus* (Linnaeus, 1758) [guttulatus-form], and *Hippocampus hippocampus* as *Hippocampus brevirostris* Schinz, 1822. However, most of recent authors classify the short-snout European seahorse as *H. hippocampus* (Linnaeus, 1758) and the long-snout one as *H. guttulatus* Cuvier, 1829 (Vasil'eva, 2007). In the absence of further data, particularly of molecular type, the most commonly used name *H. hippocampus* has been retained in the present study for the short-snouted European seahorse.

H. hippocampus is distributed along the eastern Atlantic waters, from British Isles and Wadden Sea southward to the Gulf of Guinea, including Azores, Madeira and Canary Islands, and also in the Mediterranean Sea (Lourie *et al.*, 2004). Although Spain is included within the known distribution range of *H. hippocampus*, scarce information is available on exact locations and populations abundance. The occurrence of *H. hippocampus* in Galicia (NW Spain) has never been reported (see the last updated checklist of marine fishes of Galicia by Bañón *et al.*, 2010). *H. hippocampus* is listed as Data Deficient since 2003 by IUCN, and recently has been included in the List of Spanish Wild Species under special protection. Currently, no *Hippocampus* species are included in the cata-

log of threatened species by the Regional Government of Galicia (<http://www.faunagallega.es.tl/>).

The first records of seahorse *H. hippocampus* from Galician waters are given, providing genetic and morphological identification, together with habitat characteristics, so that this species might be considered in the next updated checklist of Galicia marine fishes.

MATERIAL AND METHODS

Scuba diving surveys were carried out regularly from 2006 to 2012 in Project 'Hippocampus' with the aim to study wild seahorse populations in Galicia (NW Spain). The following sites were visited: Toralla Island (42°12.12'N; 8°47.90'W), A Guía (42°15.59'N; 8°42.24'W), Bueu (42°19.86'N; 8°46.71'W), Punta Cabalo (42°34.32'N; 8°53.05'W), Ribeira (42°33.77'N; 8°59.25'W) and Sada (43°21.27'N; 8°14.71'W). Nine adults of *H. hippocampus* were found between 2010 and 2012 and GPS position, depth of

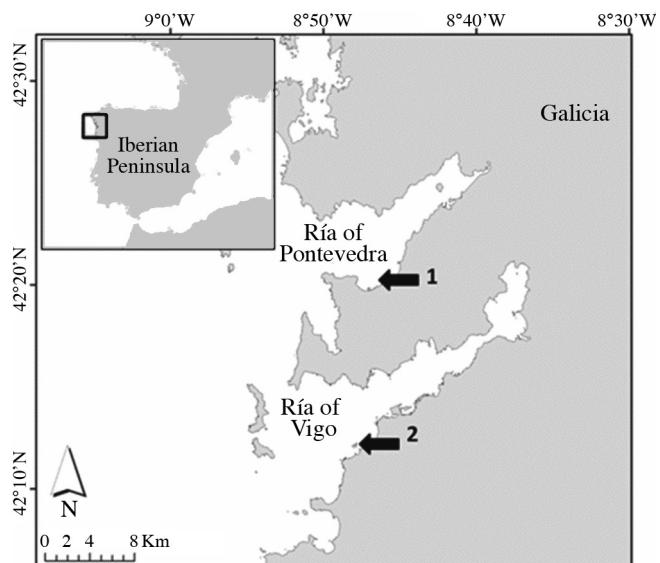


Figure 1. - Map showing the locations where seahorses *H. hippocampus* were recorded. 1: Bueu; 2: Toralla Island.

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Figure 2. - Underwater photography of an adult female (ID: N204) of *H. hippocampus* in Toralla Island.

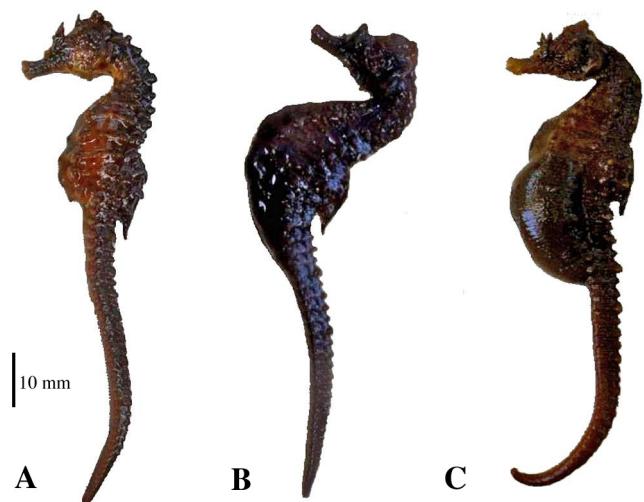


Figure 3. - Seahorse *H. hippocampus*. A: Female, Bueu, May 2011; B: Male, Bueu, Oct. 2011; C: Pregnant male, Toralla Island, Jun. 2011.

capture, habitat characteristics, sex, sexual maturity and weight were recorded. Morphometric measurements were carried out from digital photographs according to Lourie *et al.* (1999). Samples of dorsal fin were also taken for genetic analyses: microsatellite characterization (*Hgu-USC-2, 4, 13; Hcap-27, 33; Habd9*; López *et al.*, 2010), and mitochondrial cytochrome b (cytb) amplification (Woodall *et al.*, 2009) and sequencing (López *et al.*, 2010). Sequences (418 bp) were compared with GenBank cytb references of *H. hippocampus* and *H. guttulatus* (Casey *et al.*, 2004; Woodall *et al.*, 2009, 2011), also included for neighbour-joining phylogenetic analysis (MEGA v3.1; Tamura *et al.*, 2007). Cytb haplotype of *H. abdominalis* (GenBank accession no AF192640) was used as outgroup. Morphometric and genetic data were used to confirm species identity of seahorses.

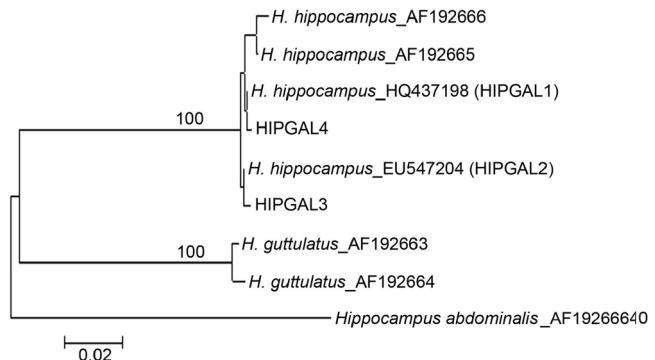


Figure 4. - Phylogenetic analysis of *H. hippocampus* cytb haplotypes from Galicia, NW Spain (HIPGAL1-4) regarding GenBank cytb sequences of seahorse species: *H. hippocampus* (AF192666 from Italy and AF192665 from UK by Casey *et al.*, 2004; EU547204 and HQ437198 were common haplotypes from a wide population sampling across Atlantic and Mediterranean coasts by Woodall *et al.* 2009; 2011); *H. guttulatus* (AF192663 and AF192664 from UK by Casey *et al.*, 2004) and *H. abdominalis* as outgroup (AF192640 from New Zealand by Casey *et al.*, 2004). Nodes indicate neighbour-joining bootstrap support.

RESULTS AND DISCUSSION

Six females and three males of *H. hippocampus* were found at 3-8 m depth in two sites: Bueu and Toralla Island (Figs 1-3; Tab. I). One of the males (N166) was pregnant when collected (June 2011) (Fig. 3C). For the same surveying period, a total of 39 and 33 *H. guttulatus* were found in Bueu and Toralla Island, respectively. Both species were morphologically distinguishable. *H. hippocampus* has a characteristic wedge-like coronet without spines on the top of the head, a short snout and a rounded trunk shape (Lourie *et al.*, 1999). Contrarily to the observations in some areas of the Mediterranean (Curtis, 2006) and Canary Islands (López *et al.*, 2010), the presence of skin filaments in seahorses of Galicia was found to be a reliable character for identifying both species.

The morphological identification was confirmed by genetic data. Four cytb haplotypes were identified (HIPGAL1-4; Fig. 4), two of them coincident with common *H. hippocampus* haplotypes previously reported in this species (GenBank accession nos EU547204 and DQ288340; Woodall *et al.*, 2009, 2011). The novel haplotypes were submitted to GenBank (HIPGAL3, KC538904; HIPGAL4, KC538905). Up to 53 diagnostic positions were detected between *H. guttulatus* and *H. hippocampus* cytb haplotypes, in agreement with the phylogenetic analysis, which clustered all the sequences obtained in this study with the *H. hippocampus* GenBank sequences (Fig. 4). Microsatellite data bring additional support to the sequence analysis, since all seahorses in this study showed alleles in the range previously reported for *H. hippocampus*, without signs of interspecific hybridization with *H. guttulatus* based on diagnostic loci (*Hgu-USC2* and *Hgu-USC13*; López *et al.*, 2010). Moreover, multilocus microsatellite genotypes confirmed different genetic identity for the nine seahorses recorded.

The low number of *H. hippocampus* occurrences suggests that wild populations of this species in Galicia are severely restricted both in abundance and distribution. Nevertheless, the occurrence of a pregnant male indicates active breeding in the area.

H. hippocampus seahorses encountered were associated to sandy bottoms covered by seagrass (*Zostera* sp.) in Bueu and a mixture of macroalgae (mostly *Sargassum* sp., *Ulva* sp., *Dictyota*

Table I. - GPS position (degrees, minutes) of capture sites, and morphological and ecological data of seahorses *H. hippocampus* recorded in Galicia (NW Spain). F: female; M: male; Ht: height; SL: standard length; TrL: trunk length; TL: tail length; HL: head length; SnL: snout length; SnL/HL: ratio snout length to head length. *: Photography in Fig. 3.

Location	Toralla Island				Bueu				
	ID	N69	N129	N166*	N204	N149	N153*	N179*	N180
Date	Jan. 2010	Mar. 2011	Jun. 2011	May 2012	Apr. 2011	May 2011	Oct. 2011	Oct. 2011	Oct. 2011
Latitude (N)	42°12.13'	42°12.14'	42°12.15'	42°12.12'	42°19.89'	42°19.88'	42°19.89'	42°19.89'	42°19.89'
Longitude (W)	8°47.89'	8°47.89'	8°47.79'	8°47.88'	8°46.59'	8°46.59'	8°46.57'	8°46.65'	8°46.65'
Sex	F	F	M	F	F	F	M	F	M
Ht (mm)	130	146	162	146	115	157	105	116	108
SL (mm)	155	168	183	171	132	181	118	134	121
TrL (mm)	49	51	44	53	39	54	36	40	36
TL (mm)	82	92	110	90	69	96	62	71	65
HL (mm)	24	24	29	28	24	31	21	24	20
SnL (mm)	9	10	9	8	9	10	8	10	n.m.
SnL/HL	0.38	0.40	0.32	0.30	0.39	0.33	0.38	0.41	n.m.
Dorsal fin rays	18	18	18	18	18	18	18	18	18
Trunk rings	11	11	11	11	11	11	11	11	11
Tail rings	37	37	37	37	37	37	37	37	37
Weight (g)	14.7	9.9	16.8	10.5	7.3	12.1	6.5	7.0	6.7
Depth (m)	4	5	6	3	4.4	3	7.5	8	8
Habitat	<i>Sargassum</i>	<i>Cystoseira</i>	<i>Sargassum</i>	<i>Ulva, Sargassum</i>	<i>Zostera</i>	<i>Zostera</i>	<i>Zostera</i>	<i>Zostera</i>	<i>Zostera</i>

sp. and *Chondrus* sp.) and seagrass (*Zostera* sp.) in Toralla Island. As in Toralla Island, *H. hippocampus* is generally associated to habitats dominated by macroalgae (Curtis and Vincent, 2005). On the contrary, even the presence of several species of macroalgae in Bueu, all individuals were attached to *Zostera* sp.

In conclusion, the presence of *H. hippocampus* in Galician waters is the northernmost Spanish record reported for the species. We propose that *H. hippocampus* and *H. guttulatus* be included in the List of Threatened species in Galicia as a priority action for conservation purposes of both species.

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